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REQUEST FOR RESEARCH GRANT APPLICATIONS: (RFA)

RFA-85-HL-28-L

ALPHA-1-PROTEINASE INHIBITOR DEFICIENCY AND EMPHYSEMA - MOLECULAR STUDIES

National Heart, Lung, and Blood Institute
Application receipt date: December 16, 1985

The Division of Lung Diseases invites grant applications for a single competetion for support of research on synthesis and secretion of alpha-1-proteinase inhibitor (A1-Pi, also known as alpha-1-antitrypsin) which is believed to provide protection for the lung against proteolytic breakdown, thereby preventing the development of emphysema. The long term goal of this program is to generate information that will provide a basis for attempts to increase the level of this protein in PiZZ individuals through pharmacologic manipulation or gene therapy. The specific objectives of this RFA program are to elucidate the mechanisms of regulation of transcription and translation of the A1-Pi gene, the post-translational modification, as well as secretion and turnover of the protein in PiZZ individuals and compare these processes to those in individuals with normal levels of the antiprotease. Among the disciplines and expertise that may be appropriate for this research program are molecular biology, cell biology, biochemistry, and physiology.

Applications should be prepared and submitted in accordance with the objectives and requirements described in the following sections.

I. PURPOSE
II. BACKGROUND
III. OBJECTIVES AND SCOPE
IV. EXCLUSIONS
V. MECHANISM OF SUPPORT
VI. REVIEW PROCEDURES AND CRITERIA
VII. METHOD OF APPLYING

We hope this RFA and participation in this program will be of interest to you.

Zakir Bengali

Zakir H. Bengali, Ph.D.
Airways Diseases Branch
Division of Lung Diseases
National Heart, Lung,
and Blood Institute

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NATIONAL HEART, LUNG, AND BLOOD INSTITUTE*

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I. PURPOSE

The Division of Lung Diseases invites grant applications for a single competetion for support of research on synthesis and secretion of alpha-1-proteinase inhibitor (A1-Pi, also known as alpha-1-antitrypsin) which is believed to provide protection for the lung against proteolytic breakdown, thereby preventing the development of emphysema. The long term goal of this program is to generate information that will provide a basis for attempts to increase the level of this protein in PiZZ individuals through pharmacologic manipulation or gene therapy. The specific objectives of this RFA program are to elucidate the mechanisms of regulation of transcription and translation of the A1-Pi gene, the post-translational modification as well as secretion and turnover of the protein in PiZZ individuals, and to compare these processes to those in individuals with normal levels of the antiprotease.

II. BACKGROUND INFORMATION

The current understanding of emphysema evolved from an observation, made in the early 1960's, that 90% of individuals with severe functional deficiency of A1-Pi usually develop emphysema. It is hypothesized that the destructive changes in the lung associated with emphysema are mediated by increased activity of elastase due to an imbalance between the enzyme and its inhibitor, A1-Pi. It has been shown that PiZZ patients with severe emphysema have little or no antielastase activity but have measurable amounts of neutrophil elastase in their bronchoalveolar lavage fluid. Normal individuals, on the other hand, have substantial amounts of the antielastase and almost no free elastase activity in their bronchoalveolar lavage fluid under the same conditions. Individuals with circulating levels of A1-Pi that are less than 15 percent of normal have been found to be susceptible to the development of emphysema at an early age.

*The programs of the Division of Lung Diseases, National Heart, Lung, and Blood Institute are identified in the Catalog of Federal Domestic Assistance, number 13.838. Awards will be made under the authority of the Public Health Service Act, Section 301 (42 USC 241) and administered under PHS grant policies and Federal regulations, most specifically 42 CFR Part 52 and 45 CFR Part 74. This program is not subject to the intergovernmental review requirements of Executive Order 12372, or to Health Systems Agency Review.

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The gene system responsible for the synthesis of A1-Pi in the human is autosomal and allelic. There are several phenotypic polymorphic variants associated with these codominant alleles at the Pi locus, the normal being designated PiM. The defective phenotype is inherited through an autosomal recessive trait, the allele being referred to as PiZZ. Some individuals have a rare genetic condition, a null mutation associated with the A1-Pi gene, which results in nonexpression of the gene.

Although the deficient ZZ phenotype contains lower amounts of the A1-Pi activity than the normal MM phenotype, the molecular weight, carbohydrate content and specific activity of the protease inhibitor from the two phenotypes are found to be very similar. It would therefore appear that the impact of the mutational change, resulting in the deficient circulating levels of the protein, might be at some post-translational step. Comparative sequence studies of A1-Pi from PiZZ and PiMM phenotypes show that, due to a point mutation, a single amino acid replacement of glutamic acid in A1-Pi$_{(MM)}$ by lysine in A1-Pi$_{(ZZ)}$ at position 342 has occurred. In PiZZ individuals, inclusion bodies consisting mainly of an insoluble polymerized form of A1-Pi are found within the rough endoplasmic reticulum of hepatocytes. The sugar content of this sequestered protein is found to be different from that of either plasma A1-Pi$_{(MM)}$ or A1-Pi$_{(ZZ)}$. Although these observations suggest that the mutational change has an impact on processes that lead to exocytosis of this protein from producer cells, the biochemical and biophysical nature of the defective A1-Pi and reasons for its sequestration in cells have not been studied adequately. Delineation of the biochemical mechanisms involved in the inability of aberrant A1-Pi to be secreted, should help understand the functional deficiency of A1-Pi in PiZZ and other phenotypes and permit formulation of approaches for correcting the deficiency to provide protection against familial emphysema.

III. OBJECTIVES AND SCOPE

The objective of this RFA is to promote studies of the detailed processes involved in the expression of the A1-Pi gene, and in the regulation of secretion and turnover of the gene product in emphysema-prone PiZZ individuals, with the ultimate goal of generating data and concepts useful for developing pharmacologic or genetic modalities for the prevention of emphysema.

Some issues relevant to the objectives of this RFA are cited below in order to provide a perspective of the scope of research that would meet the goals of this program.

A. Molecular Biology of A1-Pi in PiZZ

Although the genes coding for PiZZ and normal A1-Pi have been cloned and characterized, very little is known about the mechanisms and regulation of transcription, post-transcriptional processing of

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RNA, translation, as well as of post-translational modifications, glycosylation, exocytosis, and turnover of the protein. Also, endogenous and possible exogenous modulators of such processes have not been explored. These issues need to be addressed in the ZZ genotype, not only in the primary cell for A1-Pi production but also in other human cell types such as the bone marrow stem cells of the hematopoietic system. This information would be helpful to design approaches for gene therapy for prevention of emphysema in PiZZ and PiNull phenotypes, a long term goal of this RFA. Since gene therapy procedures will most likely introduce the A1-Pi gene in an extrachromosomal state, it will also be necessary to understand the simultaneous expression of the native Z gene and the externally introduced normal gene in cells expressing the antiprotease gene at various levels. Although directed vector systems would be designed for the introduction of such a gene in targeted cells, it is likely that other cells might also be 'infected'. It is therefore necessary to understand the biological consequences of various levels of expression of the A1-Pi gene in cells other than the primary cell for its expression.

B. Production of A1-Pi by Mononuclear Phagocytes

It has been shown that mononuclear phagocytes produce A1-Pi, though in much smaller amounts than hepatocytes. This could be due to differences in regulation of the antiprotease gene in these two cell types. Moreover, monocytes, which are the precursor cells for mononuclear phagocytes, produce A1-Pi in even smaller amounts. However, their ability to produce the antiprotease increases with differentiation to the phagocyte stage. The modulation in expression of the A1-Pi gene as related to differentiation needs to be studied.

C. Biochemical Basis for A1-Pi Deficiency in PiZZ Phenotype

A1-Pi cDNA for the Z gene has been cloned and sequenced and the amino acid sequence of the protein has been derived from it. It has been established that the PiZZ associated defect in A1-Pi is due to a point mutation resulting in substitution of glutamic acid for lysine at position 342. How this single amino acid substitution affects the structure and possibly the glycosylation and secretion of the mutant A1-Pi remains to be understood.

Increased sialylation has often been found to be associated with increased secretion of this protein during acute phase response such as inflammation. The mechanism of secretion of A1-Pi and the role of glycoside side chains in the process, as well as the physiological signals involved in triggering increased synthesis and secretion are yet to be understood.

Studies are needed to establish whether the processes involved in the secretion of A1-Pi would be amenable to pharmacologic intervention, since this will open possibilities for enhancing the plasma levels of the antiprotease in PiZZ individuals.

It is not required that all of these topics are dealt with in a single proposal, nor are the types of issues to be addressed restricted to those described above. Investigators are encouraged to consider other relevant questions and devise multidisciplinary investigations that would expand our understanding of all aspects of A1-Pi gene expression in deficient and normal individuals and lead to approaches for pharmacologic or genetic manipulation of these processes for the prevention of emphysema.

IV. EXCLUSIONS

Proposals dealing with oxidative inactivation of A1-Pi, or interaction of A1-Pi with elastase, or exclusively with nonhuman systems will not be considered responsive to the goals of this RFA. Also, studies of liver disease associated with A1-Pi deficiency, or those concerned with development of new methodology will not be supported under this RFA program.

V. MECHANISM OF SUPPORT

The support mechanism for this program will be the traditional, individual research grant. Although approximately Ø800,000 (direct and indirect costs) for this program is included in the financial plans for fiscal year 1986, award of grants pursuant to this RFA is contingent upon receipt of funds for this purpose. It is anticipated that approximately six to eight grants will be awarded under this program. The specific amount to be funded will, however, depend on the merit and scope of the applications received and the availability of funds. Since a variety of approaches would represent valid responses to this announcement, it is anticipated that there will be a range of costs among individual grants awarded. While multidisciplinary approaches are encouraged, it is not the intent of this announcement to solicit proposals for large studies encompassing a variety of independent projects. Upon initiation of the program, the Division of Lung Diseases will sponsor periodic meetings to encourage exchange of information among investigators who participate in this program. In the preparation of the budget for the grant application, applicants should request travel funds for a one-day meeting each year, most likely to be held in Bethesda, Maryland. Applicants should also include a statement in their applications indicating their willingness to participate in these meetings.

Applicants are requested to furnish their own estimates of the time required to achieve the objectives of the proposed research project; however, the award period for this activity should not exceed 5 years. At the end of the initial award period, renewal applications may be submitted for further competitive review through the regular grant program of the NIH. It is anticipated that support will begin in June, 1986. However, depending on availability of funds, a later start date could be authorized.

The current policies and requirements that govern the research grant

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programs of the National Institutes of Health will prevail, including the requirement for cost sharing.

VI. REVIEW PROCEDURES AND CRITERIA

Review Method. All applications submitted in response to this RFA will be reviewed for scientific and technical merit by an initial review group, which will be convened by the Division of Extramural Affairs, NHLBI, solely to review these applications. Upon receipt, applications will be reviewed for their responsiveness to the objectives of this RFA. If an application is judged unresponsive, the applicant will be contacted and given an opportunity to withdraw the application or to have it considered for the regular grant program of the NIH.

If a proposal submitted in response to this RFA is identical to a research grant application already submitted to the NIH for review, the applicant will be asked to withdraw the pending application before the new one is accepted. Simultaneous submission of identical applications will not be allowed.

Review Criteria. The factors to be considered in the evaluation of scientific merit of each application will be similar to those used in the review of traditional research project grant applications including the novelty, originality, and feasibility of the approach; the training, experience, and research competence of the investigator(s); the adequacy of the experimental design; the suitability of the facilities; and the appropriateness of the requested budget to the work proposed.

VII. METHOD OF APPLYING

Letter of Intent. Prospective applicants are asked to submit a one-page letter of intent that includes a brief synopsis of the proposed research and identification of any other participating institutions. Such letters are requested for the purpose of obtaining an indication of the number and scope of applications to be received. A letter of intent is not binding, and it will not enter into the review of any application subsequently submitted, nor is it a necessary requirement for application. This letter should be received no later than October 15, 1985, and sent to:

Charles L. Turbyfill, Ph.D.
Review Branch
Division of Extramural Affairs
National Heart, Lung, and Blood Institute
National Institutes of Health
Westwood Building, Room 553
Bethesda, Maryland 20205
Telephone: 301/496-7351

Format for Applications: Submit applications on form PHS 398, the application form for the traditional research project grant. This form is available in an applicant institution's office of sponsored research.

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Use the conventional format for research project grant applications and ensure the points identified in the section on "Review Procedures and Criteria" are fulfilled. To identify the application as a response to this RFA, check "yes" on item Δ2 of page 1 of the application and enter the title "ALPHA-1-PROTEINASE INHIBITOR DEFICIENCY AND EMPHYSEMA - MOLECULAR STUDIES" and the RFA number 85-HL-28-L.

Application Procedure. Send or deliver the completed application and four (4) signed, complete photocopies of it to:

Division of Research Grants
Westwood Building, Room 240
National Institutes of Health
Bethesda, Maryland 20205

Send two additional copies of the application to Dr. Charles Turbyfill at the address listed under Letter of Intent.

Applications must be received by December 16, 1985. An application not received by this date will be considered ineligible.

Timetable.		
	Letter of Intent	October 15, 1985
	Application Receipt Date	December 16, 1985
	Review by National Advisory Council	May 22-23, 1986
	Anticipated Award Date	June 1, 1986.

Inquiries. Inquiries regarding this announcement may be directed to the program administrator:

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